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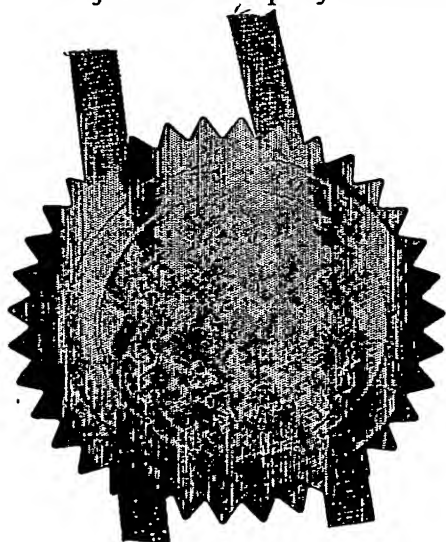
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UNIVERSITY COLLEGE LONDON
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Patents ADP number (if you know it)

798652013

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

USE OF PHOTSENSITISATION

5. Name of your agent (if you have one)

J. A. KEMP & CO.

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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Patents Form 1/77

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Description	14
Claim(s)	3
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11. I/We request the grant of a patent on the basis of this application.

Signature(s)

J.A. KEMP & CO.

Date 9 OCTOBER 2003

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USE OF PHOTSENSITISATION

The present invention relates to a composition comprising a conjugate of a
5 photosensitizer and a bacteriophage, particularly a staphylococcal bacteriophage,
known as a staphylophage. The invention also relates to the use of the conjugate in a
method of photodynamic therapy.

Background

10 The use of antimicrobial agents to counter bacterial infections is becoming
increasingly ineffective, due to the rapid emergence of antibiotic resistance amongst
many species of pathogenic bacteria. One such pathogen is *Staphylococcus aureus* (*S.*
aureus), which characteristically causes skin infections such as boils, carbuncles and
impetigo, as well as infecting acne, burns and wounds. If the infecting organism is a
15 toxic strain, such infections, or colonised tampons, may give rise to a life-threatening
toxaemia known as toxic shock syndrome. The organism may also gain access to the
bloodstream from these infections, or from foreign bodies such as intravenous
catheters, and so cause infections at other sites, such as endocarditis, osteomyelitis,
meningitis and pneumonia.

20 A number of bacteria are responsible for infection of skin and wounds, for
example, coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, streptococci,
Corynebacterium spp., *E. coli*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*,
Enterobacter aerogenes, *Propionibacterium acnes* and *Bacteroides spp.*,
Pseudomonas aeruginosa and *Peptostreptococcus spp.* Increasingly, these bacteria
25 are showing resistance to antibiotic treatment.

In particular, resistant strains of *S. aureus* have emerged. Methicillin-resistant
S. aureus (MRSA) was first reported in 1961 (Jevons, M. (1961) British Medical
Journal, 1, 124-5), and these strains are now a major cause of hospital-acquired
infection throughout the world, as well as being prevalent in many nursing and
30 residential homes. This poses an alarming challenge to healthcare, causing significant

infection and morbidity of hundreds of patients in the UK each year (Ayliffe *et al*, J Hosp Infect (1988), 39, 253-90).

Since the first report of MRSA, these organisms have demonstrated resistance to a wide variety of antimicrobials including erythromycin, aminoglycosides, 5 tetracyclines, trimethoprim, sulphonamides and chloramphenicol. MRSA strains have developed that are only susceptible to a single class of clinically-available antibiotics: the glycopeptides such as vancomycin and teicoplanin. However, resistance is developing even to these, as strains tolerant to high levels of vancomycin have now been reported (Hiramatsu, K. (1998) American Journal of 10 Medicine, 104, 7S - 10S). At present, the management of patients with MRSA infections usually involves the administration of antimicrobial agents and again, there is evidence of the development of resistance to many of the agents used.

Due to the emergence of strains which are resistant to virtually all currently-available antimicrobials, MRSA is now a serious threat to health. The term MRSA 15 itself now more accurately applies to methicillin and multiple antimicrobial-resistant *S. aureus*.

Certain strains of MRSA have been found to spread rapidly not only within hospitals, but also between them. These strains have been termed epidemic MRSA (EMRSA). Since the first EMRSA strain (EMRSA-1) was reported in 1981, 17 20 distinct EMRSA strains have been identified, all of which are resistant to a number of antimicrobials. Recently, the two most prevalent strains have been EMRSA-15 and -16, which account for 60-70% of the 30000 MRSA isolates reported (Livermore, D (2000) Int. J. Antimicrobial Agents, 16, S3 - S10).

It is clear from the above that alternative methods of countering bacterial 25 infection, particularly infection with MRSA, are urgently required.

One approach has been to employ a light-activated agent to achieve lethal photosensitization of the organism. This involves treating the organism with a light-activatable chemical (photosensitizer) which, upon irradiation with light of a suitable wavelength, generates cytotoxic species, resulting in bacteriolysis. This technique has 30 been used to achieve killing of a wide range of bacteria, including *S. aureus* and

MRSA strains, *in vitro* using toluidine blue O (TBO) and aluminium disulphonated phthalocyanine (AlPcS₂) as photosensitizers. Neither photosensitizer nor laser light alone exerted a bacteriocidal effect (Wilson *et al*, (1994) J Antimicrob Chemother 33, 619-24). In a subsequent study, 16 strains of EMRSA were found to be
5 susceptible to killing by low doses of red light (674 nm) in the presence of AlPcS₂ (Griffiths *et al*, (1997) J Antimicrob Chemother, 40, 873-6). At higher light doses, 100 % killing was achieved.

Photodynamic therapy (PDT) is the application of such an approach to the treatment of disease. It is an established procedure in the treatment of carcinoma and
10 forms the basis of a means of sterilising blood products. It has only been more recently that the application of PDT to the treatment of infectious diseases has been evaluated. For example, haematoporphyrins in conjunction with an argon laser have been used to treat post-neurosurgical infections and brain abscesses (Lombard *et al*, (1985), Photodynamic Therapy of Tumours and other Diseases, Ed. Jori & Perria).

15 One potential problem associated with PDT of infectious diseases is its lack of specificity. Hence, if the photosensitizer binds to, or is taken up by, a host cell, as well as the target organism, then subsequent irradiation may also lead to the death of the host cell. A way to overcome this is by the use of targeting compounds: that is, any compound that is capable of specifically binding to the surface of the pathogen.

20 Several targeting compounds have previously been shown to be successful in eliminating specific strains of bacteria when they were conjugated to a photosensitizer and used in PDT. For example, immunoglobulin G (IgG) has been used to target *S. aureus* Protein A (Gross *et al* (1997), Photochemistry and Photobiology, 66, 872-8), monoclonal antibody against *Polyphryomonas gingivalis*
25 lipopolysaccharide (Bhatti *et al* (2000), Antimicrobial Agents and Chemotherapy, 44, 2615-8) and poly-L-lysine peptides against *P. gingivalis* and *Actinomyces viscosus* (Soukos *et al* (1998), Antimicrobial Agents and Chemotherapy, 42, 2595-2601). A monoclonal antibody conjugated via dextran chains to the photosensitizer tin (IV) chlorin e6 (SnCe6) was selective for killing *P. aeruginosa* when exposed to light at
30 630nm, leaving *S. aureus* unaffected (Friedberg *et al* (1991), Ann N Y Acad Sci,

618, 383-393).

The present inventors have used IgG conjugated to SnCe6 to target EMRSA strains 1, 3, 15 and 16 (Embleton *et al* (2002), J Antimicrob Chemother, **50**, 857-864), achieving higher levels of killing than the photosensitizer alone, and selectively
5 killing the EMRSA strains in a mixture with *S. sanguis*. However, a limitation of IgG is that only strains of *S. aureus* expressing Protein A can be targeted. Hence alternative targeting agents that can target any *S. aureus* strain are desirable.

Bacteriophage are viruses that infect certain bacteria, causing them to lyse and hence effecting cell death. They have been proposed as antibacterial agents in their
10 own right. However, one of the problems with using staphylococcal bacteriophage (termed staphylophage) in the treatment of *S. aureus* disease is their restricted host range. Although there are polyvalent staphylophage which can lyse many *S. aureus* strains, other strains are resistant and hence bacteriophages alone could not provide an effective method of killing all strains of *S. aureus*.

15 The present inventors have now found that although some bacteriophage will only kill a limited range of bacteria, they will bind to a broader range of bacteria and can serve as an effective, targetted delivery system for photosensitizers.

The present inventors have found that when a bacteriophage is linked to a photosensitizer, the photosensitizer-bacteriophage conjugate formed is highly
20 effective in killing bacteria when irradiated with light of a suitable wavelength.

Bacteriophage-photosensitiser conjugates could be used to treat a broad range of bacterial skin and wound infections. The most frequently isolated organisms from skin and wound infections are: coagulase-negative *Staphylococcus*, *Staph. aureus*, streptococci, *Corynebacterium spp.*, *E coli*, *Klebsiella aerogenes*, *Klebsiella*
25 *pneumoniae* and *Enterobacter aerogenes*. *Propionibacterium acnes* and *Bacteroides spp.*, *Pseudomonas aeruginosa* and *Peptostreptococcus spp.*.

In particular, conjugates of photosensitiser and staphylophage can be used in a method of photodynamic therapy against strains of staphylococcus, particularly against MRSA and EMRSA.

The invention provides a composition comprising a photosensitizing compound (photosensitizer) linked to a bacteriophage to form a photosensitizer-bacteriophage conjugate. The bacteriophage may be a staphylococcal phage, and is preferably a staphylophage effective against *Staphylococcus aureus*, particularly MRSA or EMRSA. The composition may be used in a method of photodynamic therapy.

The bacteriophage is preferably linked to the photosensitizer using a covalent linkage. The photosensitiser and/or the bacteriophage contain or may be modified to contain groups which can be covalently crosslinked using chemical or photoreactive reagents, to produce crosslinked bonds, for example thiol-thiol crosslinking, amine-amine crosslinking, amine-thiol crosslinking, amine-carboxylic acid crosslinking, thiol-carboxylic acid crosslinking, hydroxyl-carboxylic acid crosslinking, hydroxyl-thiol crosslinking and combinations thereof.

The photosensitizer is suitably chosen from arianor steel blue, toluidine blue O, crystal violet, methylene blue, azure blue cert, azure B chloride, azure 2, azure A chloride, azure B tetrafluoroborate, thionin, azure A eosinate, azure B eosinate, azure mix sicc., azure II eosinate, haematoporphyrin HC1, haematoporphyrin ester, aluminium disulphonated phtalocyanine and chlorins, preferably tin (IV) chlorin e6 (SnCe6).

The invention is directed to killing bacteria using the above-described conjugates. The bacteriophage used in the conjugate may be selected according to the particular bacteria to be killed, in order to arrive at the conjugate most effective against the particular infecting bacteria. In a preferred embodiment, the infecting bacterium is MRSA or EMRSA and the conjugate includes the staphylococcal phage 75.

Table 1 below shows some examples of bacteria-bacteriophage pairs, although many more examples exist. It is relatively straightforward to isolate novel bacteriophage and/or to adapt bacteriophage to the target bacteria. The specificity of the treatment can be modified as required by using monovalent bacteriophage,

polyvalent bacteriophage or combinations of monovalent bacteriophage or combinations of monovalent and polyvalent bacteriophage.

Bacterium	Bacteriophage
5 <i>Staphylococcus aureus</i>	53, 75, 79,80,83, ϕ 11, ϕ 12, ϕ 13, ϕ 147, ϕ MR11
<i>Staphylococcus epidermidis</i>	48, 71, numerous (182 different phage)
<i>Staphylococcus spp</i>	phi ϕ 812, SK311, phi ϕ 131, SB-I and U16
<i>Streptococcus spp</i>	C ₁ , SF370.1, SP24,SFL, various
<i>Corynebacterium spp</i>	ϕ 304L ϕ 304S, ϕ 15, ϕ 16, 782
10 <i>Klebsiella aerogenes</i> and	
<i>Klebsiella pneumoniae</i>	P1clr100KM
<i>E coli</i>	P1, T1, T3, T4, T7 MS2
<i>Enterobacter aerogenes</i>	Various, P1, M13
<i>Pseudomonas aeruginosa</i>	UNL-1, ACQ, UT1, tbaID3, E79, F8 & pf20 B3, F116,
15 G101,	B86, T7M, ACq, UT1, BLB
<i>Propionibacterium acnes</i>	Various
<i>Bacteroides spp</i>	B40-8
Numerous Gram negative bacteria P1	Various

20 The composition of the invention suitably comprises from 0.05 to 200 μ g/ml of the photosensitizer, preferably from 0.5 to 15 μ g/ml, more preferably from 13.5 to 14.8 μ g/ml. The amount of the bacteriophage in the composition is suitably from 1×10^6 to 1×10^9 pfu, preferably from 6.5×10^6 to 7.3×10^7 pfu. It is preferred that the photosensitiser and the bacteriophage are present in the composition in a ratio of

25 from 1 phage to from 0.5 to 100 photosensitiser molecules.

The composition of the invention may further comprise a source of calcium ions, for example calcium chloride or calcium carbonate. The Ca²⁺ ions are suitably present in an amount of from 5 to 15mM, preferably about 10mM.

30 The composition may further comprise one or more ingredients chosen from buffers, salts for adjusting the tonicity, antioxidants, preservatives, gelling agents and remineralisation agents.

The invention further provides a method of killing bacteria, comprising

- (a) contacting an area to be treated with the composition of the invention such that any bacteria in the area take up the photosensitizer-bacteriophage conjugate; and
- (b) irradiating the area with laser light at a wavelength absorbed by the photosensitizer.

Suitably the bacteria are as set out above, preferably *Staphylococcus aureus*, more preferably MRSA or EMRSA.

In the method of the invention, any light source that emits light of an appropriate wavelength may be used. The wavelength of the light is selected to correspond to the absorption maximum of the photosensitiser and to have sufficient energy to activate the photosensitiser. The wavelength of the light emitted by the light source may be from 400 to 1060nm. A suitable laser may have a power of from 1 to 100mW and a beam diameter of from 1 to 10mm. The duration of laser irradiation is suitably from one second to 15 minutes, preferably from 1 to 5 minutes, and the light dose is suitably from 5 to 333 J cm⁻², preferably from 5 to 30 J cm⁻².

The following lasers may be suitable for use in the present invention:

- Helium neon (HeNe) gas laser (632.8nm)
- Argon-pumped dye laser (500-700nm, 5W output)
- Copper vapour-pumped dye laser (600-800nm)
- Excimer-pumped dye laser (400-700nm)
- Gold vapour laser (628nm, 10W output)
- Tunable solid state laser (532-1060nm), including Sd:YAG
- Light emitting diode (LED) (400-800nm)
- Diode laser (630-850nm, 25W output), including gallium selenium arsenide.

In the method of the invention, the composition is suitably in the form of a solution or a suspension in a pharmaceutically acceptable aqueous carrier, but may be in the form of a solid such as a powder or a gel, an ointment or a cream. The

composition may be applied to the infected area by painting, spreading, spraying or any other conventional technique.

The invention further provides the use of the composition for treatment of the human or animal body. Suitably, the composition is provided for use in the treatment
5 of conditions resulting from bacterial infection, particularly by staphylococcal bacteria, more particularly by MRSA or EMRSA.

The invention may be used to treat bacterial infection, particularly by staphylococcal bacteria, more particularly by MRSA or EMRSA, to treat or prevent skin infections such as boils, carbuncles and impetigo, to treat or prevent infections
10 of acne, burns or wounds, or to treat or prevent endocarditis, osteomyelitis, meningitis and pneumonia, arising as a result of bacterial infection, or to prevent infection following an operation, such as Caesarean section.

The invention may also be used in the prevention of carriage of the bacteria by carriers who themselves show few, if any, symptoms.

15

Description of the Figures

Results from Examples 1 to 5 are presented in Figure 1, showing the effect of a phage 75-SnCe6 conjugate on different EMRSA strains.

20

EXAMPLES

Materials and Methods

25 The following media were prepared:

Nutrient Broth 2 (NB2) medium

One litre of medium was made by adding 25g of Nutrient Broth 2 (Oxoid)
(10.0 g/l Lab-Lemco powder, 10.0 g/l peptone, 5.0 g/l NaCl) to 1 litre of deionised,
30 distilled water

Tryptone Soya Broth (TSY)

One litre of medium was made by adding 39g of Tryptone Soya Broth (Oxoid) (17.0 g/l pancreatic digest of casein, 3.0 g/l papaic digest of soybean meal, 2.5 g/l glucose, 2.5 g/l di-basic potassium phosphate, 5.0 g/l NaCl) and 0.5% of yeast extract (9.8 g/l total nitrogen, 5.1 g/l amino nitrogen, 0.3 g/l NaCl) to 1 litre of deionised, distilled water. After mixing, the medium was autoclaved at 121 °C for 15 min.

Nutrient Broth 2 Top Agar

0.35 % (w/v) of Agar Bacteriological (Agar No. 1, Oxoid) was added to NB2 medium.

Nutrient Broth 2 Bottom Agar

0.7% (w/v) of Agar Bacteriological was added to NB2 medium. After autoclaving, 10 mM of CaCl₂ was added (10ml 1M CaCl₂ in 1 litre of NB2).

Columbia Blood Agar (CBA)

37.1g of Columbia Agar Base (Oxoid) (23.0 g/l special peptone, 1.0 g/l starch, 5.0 g/l NaCl, 10.0 g/l agar) was added to 1 litre of deionised, distilled water. After autoclaving, the liquid agar was allowed to cool at room temperature until cool enough to handle. 5% (v/v) defibrinated horse blood (E & O Laboratories, Scotland) was then added.

Mannitol Salt Agar (MSA)

111g of Mannitol Salt Agar (Oxoid) (75.0 g/l NaCl, 10.0 g/l mannitol, 1.0 g/l Lab-lemco powder, 10.0 g/l peptone, 0.025 g/l phenol red, 15.0 g/l agar) was added to 1 litre of deionised, distilled water.

All mixtures were autoclaved at 121 °C for 15 min. The liquid agar was then poured into plates, covered and allowed to cool overnight.

Target organisms

The organisms used in the examples were as follows, given as names and NCTC (National Collection of Type Cultures, UK) numbers:

Epidemic methicillin-resistant *S. aureus* (EMRSA)-1 (NCTC 11939)

5 EMRSA-3 (NCTC 13130)

EMRSA-15 (NCTC 13142)

EMRSA-16 (NCTC 13143)

Staphylococcus epidermidis (NCTC 11047).

All were maintained by weekly subculture on CBA.

10

Bacteriophage

Phage 75 (Public Health Laboratory Service, UK) is a serogroup F *Staphylococcal* phage, capable of infecting EMRSA-16, EMRSA-3 and weakly infecting EMRSA-15.

15

Bacteriophage propagation

Mid-exponential EMRSA-16 (300µl) was added to 15ml Falcon Tubes. Approximately 10^5 pfu of phage 75 was added to the tubes and allowed to incubate at room temperature for 30 min to allowed the phage to the bacteria. 9ml of cooled
20 molten top NB2 agar (with 10nM CaCl_2), was added to the tubes, and the mixture poured onto undried NB2 base agar plates. The plates were left to incubate at 37°C overnight.

The next morning 1 ml of NB2 with 10 mM CaCl_2 was added to each plate, and the top agar with the liquid medium was scraped into a small centrifuge tube.

25 The collected agar was then spun in a centrifuge at 15000 rpm for 15 min at 4°C. The supernatant was collected and passed through a 0.45µm (Nalgene) filter to remove any bacterial cells. The resulting solution of phage 75 was stored at 4°C.

Bacteriophage precipitation

Phage precipitation was carried out to purify the phage 75 from the NB2 medium after propagation. To 5ml of phage 75 in NB2, 1.3 ml of 5M NaCl (1M final concentration) and 0.2 ml 1x phosphate buffered saline (PBS) (8.0g NaCl, 0.2g KCl, 1.15 g Na₂HPO₄, 0.2g KH₂PO₄) were added, and 20% PEG (polyethylene glycol, Sigma) was added to the solution and stirred slowly overnight until completely dissolved. The solution was then placed on ice overnight and the next morning the solution was centrifuged at 8000rpm for 20 min at 4°C. The supernatant was removed and the remaining pellet was resuspended in 2.5ml 1x PBS, and filtered through a 0.45 µm filter.

10

Photosensitizer

The photosensitizer used was tin (IV) chlorin e6 (SnCe6) (Frontier Scientific, Lancashire, UK), which is photoactivatable at 633 nm.

15 Preparation of conjugate

2mg of SnCe6 was dissolved with stirring in 800 µl of activation buffer (0.1 M MES (2-(N-morpholino)ethanesulphonic acid) (Sigma)), 0.5 M NaCl, pH 5.5). An EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) (Sigma) solution (4mg in 1 ml activation buffer) and a S-NHS (N-hydroxysulphosuccinimide) (Fluka) solution (2.7 mg in 250 µl activation buffer) were made.

To the dissolved SnCe6, 200µl of dissolved EDC and S-NHS were added, and the mixture was left for 1 to 4 hours at room temperature with stirring to provide a stable amine-reactive intermediate. The mixture was covered in aluminium foil as SnCe6 is a light sensitive reagent. The reaction was quenched by adding 1.4µl β-mercaptoethanol (Sigma).

Experiment were carried out using the reagents at a molar ratio of SnCe6:EDC:S-NHS of 1:1:2.5.

The pH of the reactive SnCe6 mixture was neutralised to 7.0 by adding 0.7ml 1 M NaOH. 1.5ml of phage 75 was then added to the amine-reactive solution to

30

allow the amino groups on the phage to react with the carboxyl groups of the SnCe6, and then mixed for 4 to 16 hours. The reaction was quenched with 2.5 µl ethanolamine (Sigma).

In the examples below, the concentration of phage 75 is 7.3×10^6 pfu/ml and
5 the concentration of SnCe6/bacteriophage-SnCe6 is 1.5 µg/ml.

Laser

The laser used was a Model 127 Stabilite helium-neon (He/Ne) laser (Spectra Physics, USA) with a power output of 35 mW. The laser emitted radiation in a
10 collimated beam, diameter 1.25 mm, with a wavelength of 632.8 nm.

Example 1

A culture of EMRSA-16 in the mid-exponential growth phase was diluted to 1×10^7 cfu/ml. 20 µl samples of the diluted bacteria were then placed into wells of a
15 96-well plate (Nunc), together with a magnetic stirrer bar.

100 µl of the phage 75-SnCe6 conjugate prepared above and 10mM calcium chloride (CaCl_2) were added to the bacteria. The contents of the wells were left to incubate at room temperature for 5 min, with stirring. Controls were performed with 100 µl 1xPBS added to the bacteria and used as a reference for experimental
20 samples. The experiment was carried out in duplicate.

After incubation, the contents of the well were directly exposed to the laser light for 5 min, with stirring, corresponding to an energy density of 21 J/cm^2 . Aluminium foil was placed in the surrounding wells to allow any escaping laser light to be reflected back into the target well. Controls were performed with no laser
25 irradiation.

After exposure to the laser, 100 µl samples were immediately taken from each well and serially diluted, from 10^{-1} to 10^{-4} , in 1 ml TSY in 1.5 ml Eppendorf tubes. Aliquots of 50 µl of each dilution were then placed and spread out on half a CBA plate. The plates were placed in a 37°C incubator overnight. The following morning

the number of survivors was counted, the average between the four sets was taken and multiplied by the appropriate dilution factor, and graphically analysed.

It was found that over 99.9% of the EMRSA-16 was killed.

5 **Example 2**

Example 1 was repeated, using EMRSA-1 in place of EMRSA-16. It was found that 99.98% of the bacteria were killed.

Example 3

10 Example 1 was repeated, using EMRSA-3 in place of EMRSA-16. It was found that over 99.99% of the bacteria were killed.

Example 4

15 Example 1 was repeated, using EMRSA-15 in place of EMRSA-16. It was found that over 99.99% of the bacteria were killed.

Example 5

20 Example 1 was repeated, using *S. epidermis* in place of EMRSA-16. It was found that over 99.99% of the bacteria were killed.

Result for Examples 1 to 5 are presented in Figure 1.

Example 6

25 Example 1 was repeated, using 10µl each EMRSA-16 and *S. epidermidis* in place of the 20µl samples of EMRSA-16. Samples were plated on MBA plates for enumeration.

It was found that over 99.99% of both bacterial strains was killed in the mixed culture.

30 **Comparitive Example**

Example 6 was repeated, firstly in the absence of conjugate, and without exposing to laser light, secondly with SnCe6 photosensitiser and exposure to laser light, and thirdly with phage 75 and without exposure to laser light.

The results for Example 6 and for the Comparative Example are presented in
5 Figure 2.

The Examples show that the conjugate is highly effective at killing all of the EMRSA strains tested. Since phage 75 is only capable of infecting EMRSA-15 and EMRSA-16, this indicates that the phage is able to successfully bind to strains it is
10 incapable of infecting, thus acting as an effective targetting agent. The attached photosensitizers then effected the killing upon laser irradiation.

Significant kills were also obtained with *S. epidermidis*, both alone and in a mixture with MRSA, indicating that the phage also bound to non-related staphylococcal strains. The phage 75-SnCe6 conjugate is useful for a variety of
15 staphylococcal infections.

CLAIMS

1. A composition comprising a conjugate of a photosensitizer and a
5 bacteriophage.
2. A composition according to claim 1, wherein the bacteriophage is a
staphylococcal bacteriophage.
3. A composition according to claim 1 or 2, wherein the photosensitizer
is covalently linked to the bacteriophage.
- 10 4. A composition according to any of claims 1 to 3, wherein the
photosensitizer is chosen from arianor steel blue, toluidine blue O, crystal violet,
methylene blue, azure blue cert, azure B chloride, azure 2, azure A chloride, azure B
tetrafluoroborate, thionin, azure A eosinate, azure B eosinate, azure mix sicc., azure
II eosinate, haematoporphyrin HC1, haematoporphyrin ester, aluminium
15 disulphonated phtalocyanine and chlorins.
5. A composition according to claim 4, wherein the photosensitizer is tin
(IV) chlorin e6 (SnCe6).
6. A composition according to any of the preceding claims, wherein the
bacteriophage is chosen from phage 53, 75, 79,80,83, ϕ 11, ϕ 12, ϕ 13, ϕ 147, ϕ MR11,
20 48, 71, ϕ 812, SK311, ϕ 131, SB-I, U16, C₁, SF370.1, SP24,SFL, ϕ 304L, ϕ 304S,
 ϕ 15, ϕ 16, 782, P1clr100KM, P1, T1, T3, T4, T7 MS2, P1, M13, UNL-1, ACQ,
UT1, tbalD3, E79, F8, p ϕ 20 B3, F116, G101, B86, T7M, ACq, UT1, BLB, and B40-
8.
7. A composition according to claim 6, wherein the bacteriophage is
25 phage 75.
8. A composition according to any of the preceding claims, wherein the
concentration of the photosensitizer is from 13.5 to 14.8 μ g/ml.
9. A composition according to any of the preceding claims, wherein the
concentration of the staphylophage is from 6.5×10^6 to 7.3×10^7 pfu/ml.

10. A composition according to any of the preceding claims, wherein the ratio of bacteriophage to photosensitizer in the composition is from 1 bacteriophage to from 0.5 to 100 molecules of photosensitiser.

11. A composition according to any of the preceding claims, which further comprises a source of Ca^{2+} ions, preferably calcium carbonate.

12. A composition according to any of claims 1 to 11, in the form of a solution in a pharmaceutically acceptable carrier.

13. A composition according to any of claims 1 to 12, wherein the composition further comprises one or more of a buffer, salt, antioxidant, preservative, gelling agent or remineralisation agent.

14. A method of killing bacteria, comprising

(a) contacting an area to be treated with a composition according to any of the preceding claims, such that any bacteria present take up the photosensitizer-bacteriophage conjugate; and

(b) irradiating the area with laser light at a wavelength absorbed by the photosensitizer.

15. A method according to claim 14, wherein the bacteria are staphylococcus, particularly MRSA or EMRSA.

16. A method according to any of claims 14 or 15, wherein the laser light is from a helium neon gas laser or gallium arsenide laser.

17. A method according to any of claims 14 to 16, wherein the laser light has a wavelength of from 400 to 1060nm.

18. A method according to any of claims 14 to 17, wherein the laser has a power of from 1 to 100mW and a beam diameter of from 1 to 10mm.

19. A method according to any of claims 14 to 18, wherein the duration of laser irradiation is from one second to 15 minutes and the light dose is from 5 to 333 Jcm^{-2} .

20. A method according to any of claims 14 to 19, wherein the composition is present in or on the area to be treated at a concentration of from 0.00001 to 1% w/v.

21. Use of a composition according to any of claims 1 to 13, for treatment of the human or animal body.

22. Use of a composition according to any of claims 1 to 13, in the manufacture of a medicament for treatment of bacterial infection.

5 23. Use according to claim 22, wherein the bacterial infection is *S. aureus*, particularly MRSA or EMRSA.

24. Use of a bacteriophage as a targeting agent in photodynamic therapy (PDT).

10 25. Use according to claim 24, wherein the bacteriophage is a staphylococcal phage.

26. A composition according to any of claims 1 to 13, substantially as described in the Examples.

27. A method according to any of claims 14 to 20, substantially as describe in the Examples.

15 28. A use according to any of claims 21 to 25, substantially as described in the Examples.

ABSTRACT

USE OF PHOTSENSITISATION

- 5 A composition comprising a conjugate of a photosensitizer and a bacteriophage is provided. The conjugate may be used to kill bacteria, particularly MRSA, in a targeted method of photodynamic therapy.

Phage at 7.3×10^6 pfu/ml

1/1

SnCe6 / Phage-SnCe6 at $1.5 \mu\text{g/ml}$

21 J/cm^2 laser light

Figure 1.

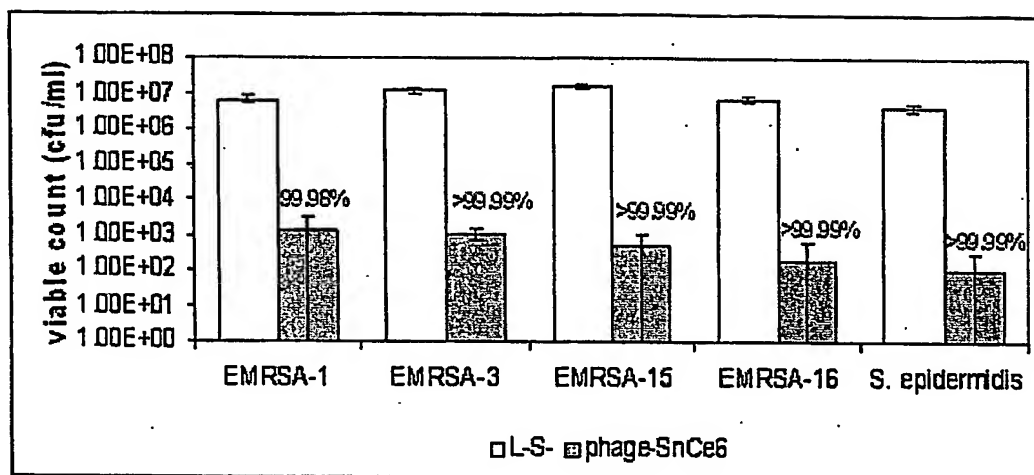
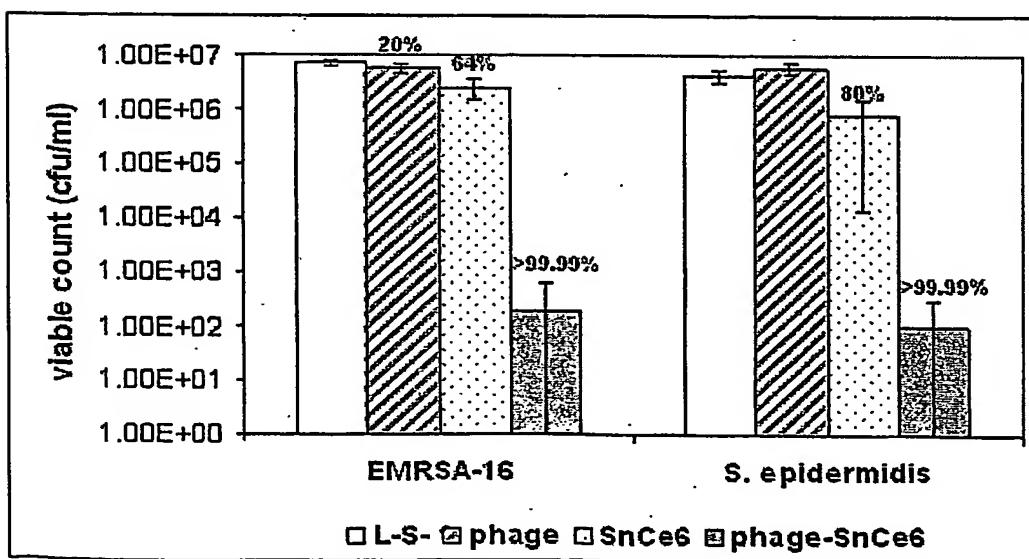


Figure 2.



L-S- = no laser light and no photosensitizer

SnCe6 = photosensitizer and laser light

Phage= bacteriophage and no laser light

Phage-SnCe6 = photosensitiser-bacteriophage conjugate and laser light